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学 位 の 種 類 博士（薬学）

学 位 記 番 号 富医薬博甲第 141 号

学位授与年月日 平成 26 年 3 月 21 日

学位授与の要件 富山大学学位規則第 3 条第 3 項該当

教 育 部 名 富山大学大学院医学薬学教育部 薬学領域 博士課程
生命薬科学専攻

学位論文題目 **Constituents of Congolese Medicinal Plants, *Garcinia huillensis*,
Securidaca longepedunculata, and *Aframomum melegueta* and
Their Preferential Cytotoxic Activity against Human
Pancreatic Cancer Cell lines
(コ ン ゴ 薬 用 植 物 *Garcinia huillensis*, *Securidaca
longepedunculata*, *Aframomum melegueta* の成分研究およびヒト膵臓
がん細胞に対する選択的細胞毒性)**

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論文内容の要旨

Cancer is a major public health problem throughout the world. Among the different forms of cancer, pancreatic cancer is the most aggressive one with a median survival rate of only 6 months and a relative 5-year survival rate of only 5.5%. It is largely resistant to the conventional forms of treatment. Human pancreatic cancer cells have been shown extreme tolerance to nutrient starvation enabling them to survive for prolong period of time even under the critically low nutrition and oxygen condition. Therefore, it has been hypothesized that the elimination of cancer cells tolerance to nutrient starvation might be one of the novel biochemical approaches (anti-austerity) for anti-cancer drug discovery.

In my study on the discovery of natural anticancer agents from Congolese medicinal plants, I have examined eight selected Congolese medicinal plants for their preferential cytotoxicity under nutrient deprived condition utilizing an anti-austerity strategy. This strategy is based on the search for agents the preferentially kill the cancer cells in nutrient deprived medium (NDM), without having toxicity to the cells in nutrient rich medium. I found that the CHCl₃ extracts of the roots of *Garcinia huillensis* and *Securidaca longepedunculata* and both the CHCl₃ and MeOH extracts of rhizomes of *Aframomum melegueta* displayed preferential cytotoxicity against PANC-1 human pancreatic cancer cells preferentially under nutrient-deprived medium (NDM) at a concentration less than 50 µg/mL. In order to identify the active constituents responsible for the observed preferential cytotoxic activity in *G. huillensis*, *S. longepedunculata*, and *A. melegueta*, I carried out further chemical investigation and the isolated compounds were tested for their preferential cytotoxicity against PANC-1 cells.

1. Constituents of *G. huillensis* and Their Preferential Cytotoxicity¹

G. huillensis, a plant of Clusiaceae family is distributed in Southern and Central Africa. It is commonly known as chikunyangulu in D. R. Congo, Musongwa in Angola and Kunguingu in Zambia. *G. huillensis* used in Congolese traditional medicine for the treatment of venereal diseases such as sores, bronchitis, angina, measles, cancer, and dermatitis.

The phytochemical investigation on this bioactive extract (PC₅₀, 17.8 µg/mL) led to the isolation of twelve anthraquinones (Chart 1). Among the compounds, damnacanthol (**1**) displayed

the most potent preferential cytotoxicity with a PC_{50} value of 4.46 μ M against PANC-1 cells. **1** also induced preferential cell death in NDM against PSN-1 human pancreatic cancer cell line with a PC_{50} value of 3.77 μ M. The treatment of **1** at different nutrient conditions against PANC-1 and PSN-1 cells suggested that **1** is highly sensitive to serum starvation. Flow cytometric analysis of **1** against PANC-1 and PSN-1 in NDM indicated that it triggered necrotic cell death (annexin V/PI⁺) in a concentration-dependent manner.

2. Constituents of *S. longepedunculata* and Their Preferential Cytotoxicity²⁻⁵

S. longepedunculata belonging to the family Polygalaceae is an important traditional folk medicine extensively used in Africa as a general remedy for several diseases such as coughs, colds, fever, backache, toothache, sleeping sickness, venereal disease, malaria, inflammation, rheumatism, snakebite, tuberculosis, ulcers, and pneumonia.

The phytochemical investigation on this bioactive extract (PC_{50} , 23.4 μ g/mL) resulted in the isolation of 40 compounds [18 new compounds including five new polymethoxylated xanthenes, 1,6,8-trihydroxy-2,3,4,5-tetramethoxyxanthone (**13**), 1,6-dihydroxy-2,3,4,5,8-pentamethoxyxanthone (**14**), 8-hydroxy-1,4,5,6-tetramethoxy-2,3-methylenedioxyxanthone (**15**), 4,6,8-trihydroxy-1,2,3,5-tetramethoxyxanthone (**16**), 4,8-dihydroxy-1,2,3,5,6-pentamethoxyxanthone (**17**) and a new benzyl benzoate [benzyl 3-hydroxy-2-methoxybenzoate (**18**)] (Chart 2), together with twelve structurally unique xanthenes which have a xanthone nucleus connected with a diphenylmethyl unit, named muchimangins A–L (**41–52**) (Chart 3). The structures of the new compounds were elucidated by analysis of their spectroscopic data. Among them, **13** and **14** displayed the most potent preferential cytotoxicity with PC_{50} of 22.8 and 17.4 μ M, in NDM against PANC-1 cells. Both compounds **13** and **14** were found to be highly sensitive to glucose starvation and induced late apoptotic/ necrotic like cell death in NDM. Diphenylmethyl-substituted hepta-oxyxanthened xanthone **44** showed the PC_{50} value of 38.9 μ M. Furthermore, it induced late apoptotic/ necrotic like cell death to PANC-1 cells. This is the first report about polymethoxylated xanthenes as anti-austeric agents.

3. Constituents of *A. melegueta* and Their Preferential Cytotoxicity¹

A. melegueta, a plant of Zingiberaceae family is distributed in western and central Africa. It is commonly known as tondolo in D. R. Congo and is used in Africa as a general remedy for several diseases such as tuberculosis, cancer, malaria, inflammation, ulcers, pneumonia, dysentery, and abdominal pain.

The phytochemical investigation on this bioactive extract (PC_{50} , 47.8 μ g/mL) led to the isolation of ten compounds (Chart 4). Among the compounds, **53** displayed the most potent

preferential cytotoxicity with PC₅₀ value 8.48 μ M against PANC-1 cells. The treatment of **53** at different nutrient conditions against PANC-1 and PSN-1 cells suggested that **53** is highly sensitive to glucose starvation and induced late apoptotic/ necrotic like cell in NDM.

Conclusion:

The CHCl₃ extracts of *G. huillensis*, *S. longepedunculata*, and *A. melegueta* from Democratic republic of Congo showed preferential cytotoxicity against PANC-1 cells. Phytochemical investigation of these extracts led to the isolation of 62 compounds including five new polymethoxylated xanthenes (**13–17**), one new benzyl benzoate (**18**) and twelve structurally unique xanthenes, muchimangins (**41–52**) which have a xanthone nucleus connected with a diphenylmethyl unit. Compounds **1** from *G. huillensis*, **13** and **14** from *S. longepedunculata* and **53** from *A. melegueta* were identified as compounds possessing potent preferential cytotoxicity against PANC-1 cells. The isolated constituents from *G. huillensis*, *S. longepedunculata*, and *A. melegueta* showed a variety structural type of compounds. In conclusion, this research demonstrates that Congolese medicinal plants and their anti-austerity agent isolated together with their derivatives are interesting candidates for drug development against pancreatic cancer.

References:

1. **Dibwe, D. F.**, Awale, S., Kadota, S., Tezuka, Y. Damnacanthol from the Congolese medicinal plant *Garcinia huillensis* has a potent preferential cytotoxicity against human pancreatic cancer PANC-1 cells. *Phytother. Res.*, **2012**, 26, 1920–1926.
2. **Dibwe, D. F.**, Awale, S., Kadota, S., Tezuka, Y. Muchimangins A–D: novel diphenylmethyl-substituted xanthenes from *Securidaca longepedunculata*. *Tetrahedron Lett.*, **2012**, 53, 6186–6190.
3. **Dibwe, D. F.**, Awale, S., Kadota, S., Morita, H., Tezuka, Y. Hepta-oxygenated xanthenes as anti-austerity agents from *Securidaca longepedunculata*. *Bioorg. Med. Chem.*, **2013**, 21, 7663–7668.
4. **Dibwe, D. F.**, Awale, S., Kadota, S., Morita, H., Tezuka, Y. Two new diphenylmethyl-substituted xanthenes from *Securidaca longepedunculata*. *Nat. Prod. Comm.*, **2014**, 9 (5), 655–657.
5. **Dibwe, D. F.**, Awale, S., Kadota, S., Morita, H., Tezuka, Y. Muchimangins E and F: novel diphenylmethyl-substituted xanthenes from *Securidaca longepedunculata*. *Tetrahedron Lett.*, **2014**, 55, 1916–1919.
6. **Dibwe, D. F.**, Awale, S., Kadota, S., Morita, H., Tezuka, Y. Muchimangins G–J: Fully-substituted xanthenes with a diphenylmethyl substituent from *Securidaca longepedunculata*. *J. Nat. Prod.*, **2014** (In press).

Anthraquinones

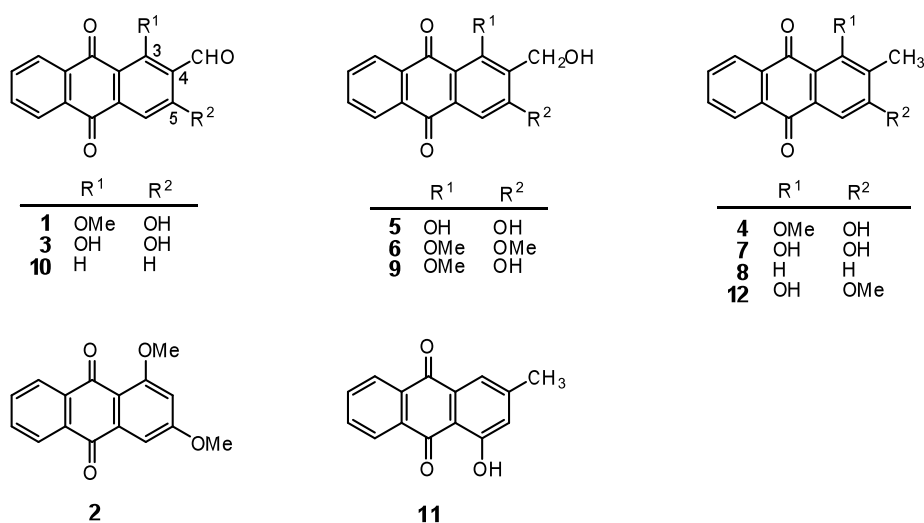
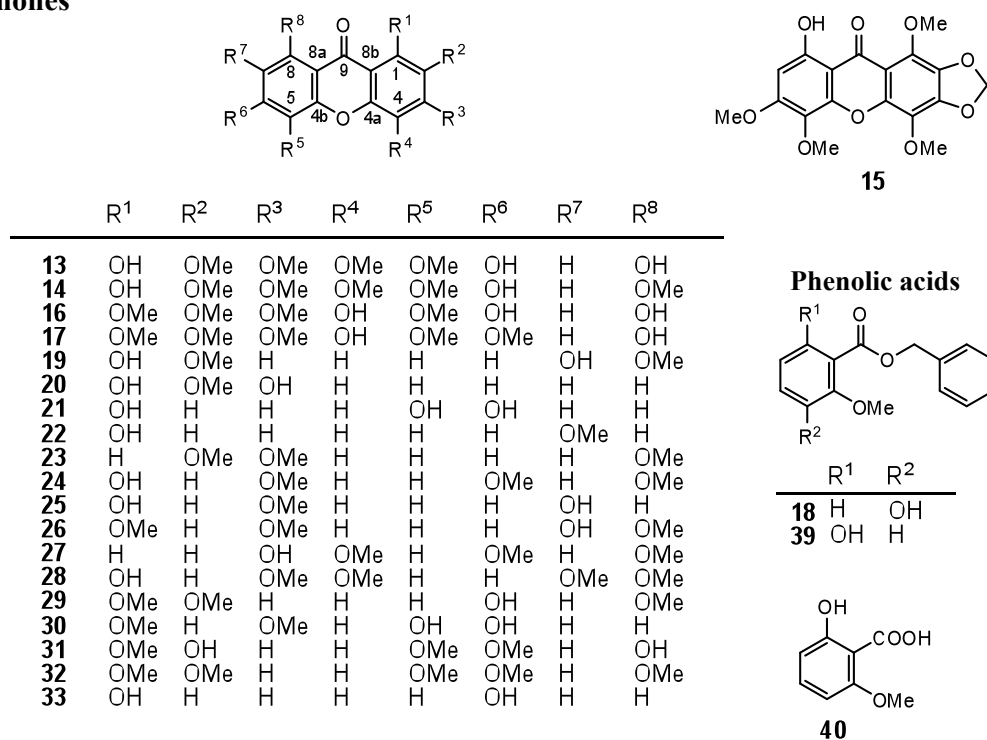


Chart 1 Structures of compounds (**1–12**) isolated from *Garcinia huillensis*

Xanthones



Benzophenones

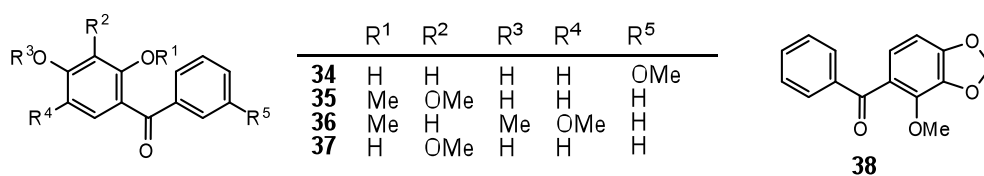


Chart 2 Structures of compounds (**13–40**) isolated from *Securidaca longepedunculata*

Muchimangins

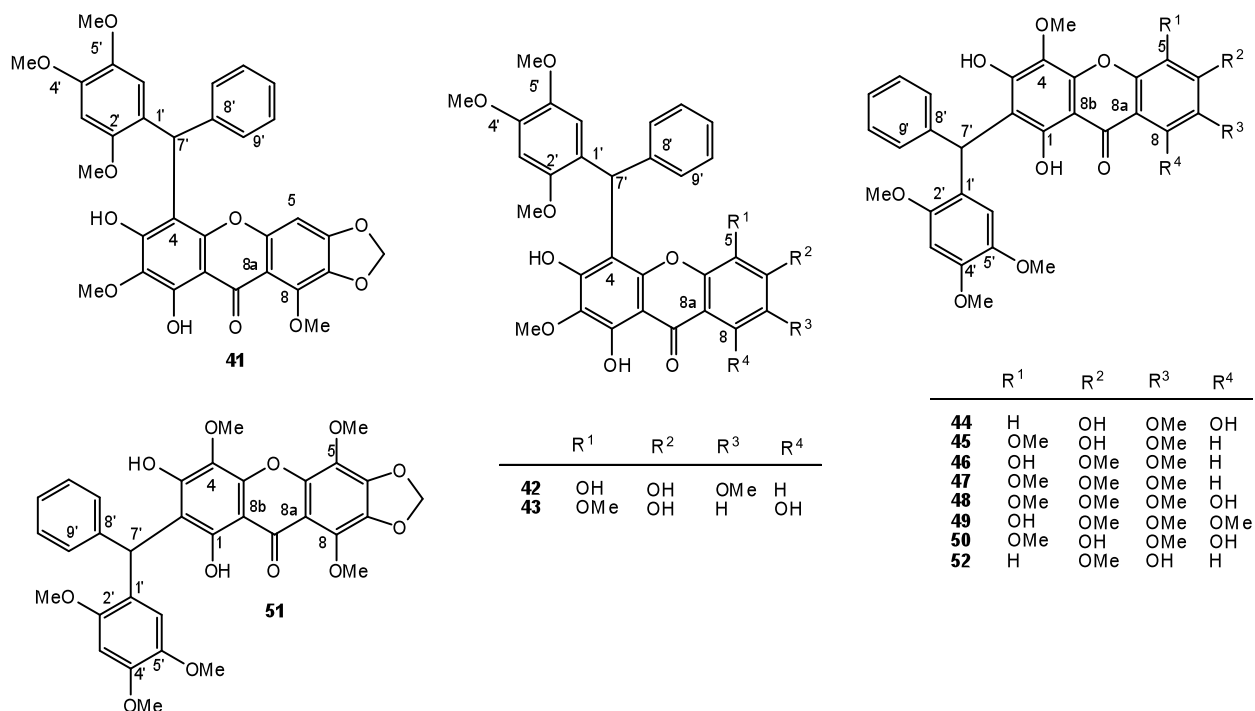
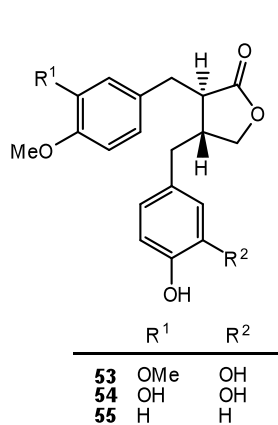
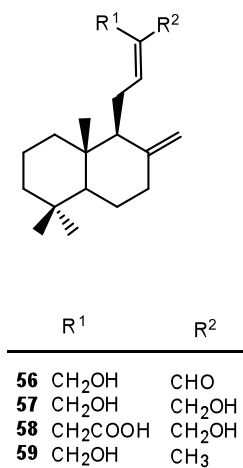


Chart 3 Structures of muchimangins (**41–52**) isolated from *Securidaca longepedunculata*

Lignans



Diterpenes



Flavonoids

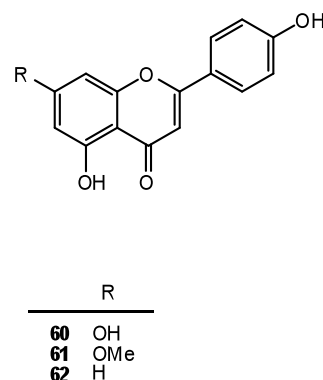


Chart 4 Structures of compounds (**53–62**) isolated from *Aframomum melegueta*